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Spectrophotometric determination of Drotaverine HCl and Mefenamic acid in tablets by chemometric methods

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ABSTRACT

Simultaneous spectrophotometric determination of Drotaverine Hcl and Mefenamic acid was performed by two chemometric methods. The chemometric methods applied were partial least-squares (PLS) and principal component regression (PCR). The methods of the chemometric analysis do not require sample pre-treatment procedure. The chemometric calibrations were prepared by measuring the absorbance values in the spectral region 220-350 nm with the intervals of 1 nm. The calibration range was found to be 2-10 µg/ml for Drotaverine Hcl, 6-30 µg/ml for Mefenamic acid. These approaches were successfully applied to quantify the two drugs in the mixture using the information included in the UV absorption spectra. The validation of the multivariate methods was realised by analysing the synthetic mixtures of Drotaverine Hcl and Mefenamic acid. The numerical calculations were performed with the 'Unscrambler 10.1 X' software. The obtained chemometric calibrations were used for the estimation of Drotaverine Hcl and Mefenamic acid in samples. By applying two techniques to synthetic mixtures and pharmaceutical preparations, the mean recoveries and the relative standard deviations were found as 99.85 % and 0.4 in PCR, 99.63% and 0.3 in PLS for Drotaverine Hcl and 100.23% and 0.33 in PCR, 100.24% and 0.34 in PLS for Mefenamic acid, respectively. The chemometrics analysis methods were satisfactorily applied to the simultaneous determination of Drotaverine Hcl and Mefenamic acid in the pharmaceutical tablet formulation.

Key words: Drotaverine Hcl, Mefenamic acid, spectrophotometry, chemometrics, partial least square, principal component regression.

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INTRODUCTION

Drotaverine hydrochloride is chemically known as 1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4-tetrahydroisoquinolene hydrochloride [1].Drotaverine hydrochloride is a highly potent spasmolytic agent. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [2]. Drotaverine hydrochloride is official in Polish pharmacopoeia [3]. A few UV-spectrophotometric [4-7] and HPLC [8- 10] methods have been reported for estimation of Drotaverine Hcl. Mefenamic acid is an orally active analgesic and anti-inflammatory drug.[11].Mefenamic acid is official in IP [12], BP [13] and USP [14]. Several UV spectrophotometric [15, 16], HPLC [17-21] and HPTLC [22] methods for the estimation of Mefenamic acid have been reported. Literature survey revealed a need for a method capable of simultaneous estimation of Drotaverine Hcl and Mefenamic acid.

The aim of this paper is to investigate the ability of PLS and PCR methods to quantify binary mixture of DROTA and MEFE with overlapping spectra and to apply the optimized models in pharmaceutical preparations. The proposed methods are simple, sensitive and reproducible method for the simultaneous estimation of Drotaverine Hcl and Mefenamic acid from combined dosage form.

In recent years, multivariate calibrations, such as classical least-squares (CLS), inverse least-squares (ILS), principal component regression (PCR) and partial least-squares (PLS) are started to apply to the analysis of the analytical data obtained in all the instrumentations [23, 24]. The same methods and their algorithms have been applied to the simultaneous spectrophotometric determination of drugs in the pharmaceutical formulation containing two or more compounds with overlapping spectra. On the other hand the chemometric calibration methods as those enumerated above have been used extensively in quantitative spectral analysis to get selective information from unselective data. The main advantages of these techniques are the following: a higher speed of processing data concerning the values of concentrations and absorbance of compounds with strongly overlapping spectra, the errors of calibration model are minimised by measuring the absorbance values at many points in the wavelength range of the zero-order and derivative spectra. Analytical methods using multivariate calibrations and their applications include the spectrophotometric, chromatographic and electrochemical for determinations of analytes in the mixtures.

MATERIALS AND METHODS

Instruments and software

Digitized UV/VIS absorbency spectra were collected using a UV-visible spectrometer 2300 Techcomp with 1 cm quartz cells. The data acquisition was made with UV solutions software at a scan rate of 1000 nm min−1 and the slit width of 2 nm. The UV spectra of mixtures were recorded over the wavelength 200-400 nm with one data point per nm. All spectral measurements were performed using blank solution as a reference. Partial least

squares regression, and principal component regression were used for chemometric analysis of data. For all calculations Unscrambler for windows (Version 10.1 X) was used.

Pharmaceutical tablet formulations

A commercial pharmaceutical formulation (Drotin™ -M)Tablet produced by Martin and Harris Lab Ltd. Mangalore, Batch no.TDMR-133 containing 80 mg DROTA and 250 mg MEFE was analysed by the proposed chemometric methods.

Standard solutions

Stock solutions of Drotaverine Hcl and Mefenamic acid of 50 mg were prepared in 100 ml volumetric flasks with methanol. The training set containing 2-10µg/ml Drotaverine Hcl and 6-30µg/ml Mefenamic acid working standard solutions were prepared by diluting the stock solutions for each drug according to its linear calibration range. Two sets of standard solutions were prepared, the calibration set contained 25 standard solutions and the prediction set contained 9 standard solutions. To a series of 10 ml volumetric flasks, aliquots of Drotaverine Hcl and Mefenamic acid solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml with methanol. UV spectra of the mixtures were recorded in the wavelength range 200-400 nm versus a solvent blank, and digitized absorbance was sampled at 1 nm intervals. All the solutions were prepared freshly and were protected from light.

Sample preparations

Twenty tablets were accurately weighed and powdered in a mortar. An amount of the powder equivalent to a tablet was dissolved in methanol in 100 ml calibrated flasks. 20ml of methanol was added and ultra sonicated for 10minutes and the volume was made up to100 ml with methanol and shake well. Then, the solution was filtered through what man filter paper No. 41and the residue was washed three times with 10 ml of solvent, and then the volume was completed to 100 ml with methanol. The resulting solution was diluted to 1:3 in a 100 ml calibrated flasks. Both techniques were applied to the prepared sample solutions.

Partial least squares (PLS) - In the UV-Vis spectra, the absorbance data (*A*) and concentration data (*C*) are mean centred to give the data matrix A_0 and vector C_0. The orthogonalised PLS algorithm has the following steps. The loading weight vector *W* has the following expression:

$$W = \frac{A_0^T C_0}{C_0^T C_0}$$

The scores and loadings are given by:

$$t_1 = \frac{A_0 W}{A_0^T C_0}$$

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$$P_1 = t_1^T t_1$$

$$q_1 = \frac{C_0^T t_1}{t_1^T t_1}$$

The matrix and vector of the residuals in *A*0 and *C*0 are:

$$A_1 = A_0 - t_1 P_1^T$$
$$C_1 = C_0 - t_1 q_1^T$$

From the general linear equation, the regression coefficients were calculated by:

$$b = W (P^T W)^{-1} q$$

$a = C_{mean} - A^T{}_{meanb}$

The built calibration equation is used for the estimation of the compounds in the samples.

Principle component regression (PCR): In the spectral work, the following steps can explain the fundamental concept of PCR. The original data obtained in absorbances (*A*) and concentrations (*C*) of analytes were reprocessed by mean-centring as *A*0 and *C*0, respectively.
Using the ordinary linear regression:

$$C = a + b \times A \qquad (6)$$

The coefficient *b* is: $b = P \times q$, where *P* is the matrix of eigenvectors and *q* is the C loadings given by $q = D \times T^T \times A_0$. Here, T^T is the transpose of the score matrix T. D is a diagonal matrix having on components the inverse of the selected Eigen values. Knowing *b* one can easily find *a* using the formula $a = C\text{mean} \times A^T_{mean} \times b$, where A^T_{mean} represents the transpose of the matrix having the entries of the mean absorbance values, and C_{mean} is the mean concentration of the calibration set.

RESULTS AND DISCUSSIONS

The absorption spectra of DROTA and MEFE solutions in methanol recorded between 200 and 400 nm were shown in Fig.1. The two drugs show an overlap in their absorption.

Experimental design of sample sets

Calibration and test sets for two component systems were designed according to factorial principle five-level factorial design was used to produce a calibration set (Training step) of 25 samples. Calibration spectra are shown in Fig.2. A three-level set was derived to produce a prediction set (Validation step) of nine samples. Prediction spectra are shown in Fig.3.The compositions of the used calibration and Validation sets are summarized in Tables.1 & 2 respectively.

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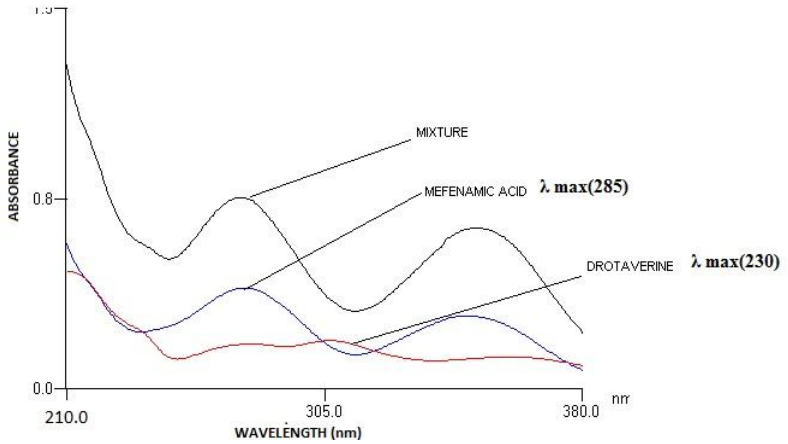

Fig.1: Overlaid UV spectra of Drotaverine Hcl, Mefenamic acid and Mixture

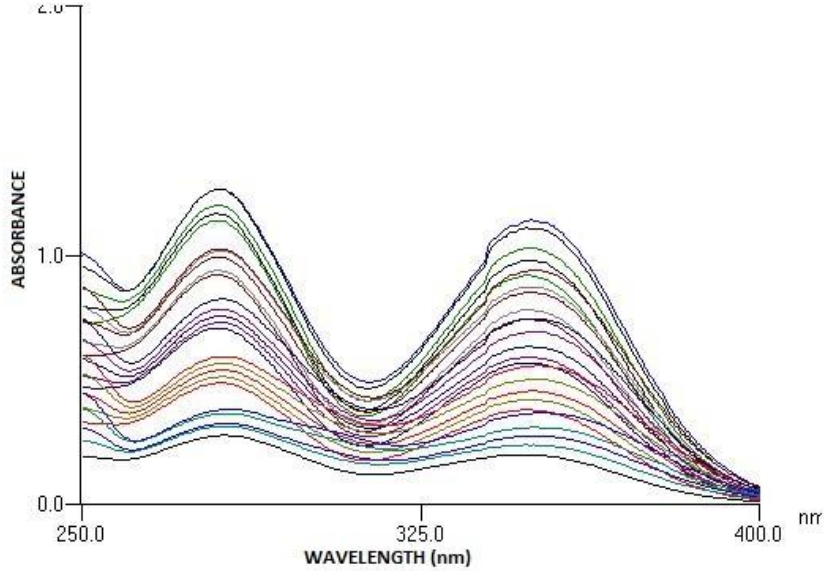

Fig. 2: Calibration spectra of Drotaverine Hcl and Mefenamic acid

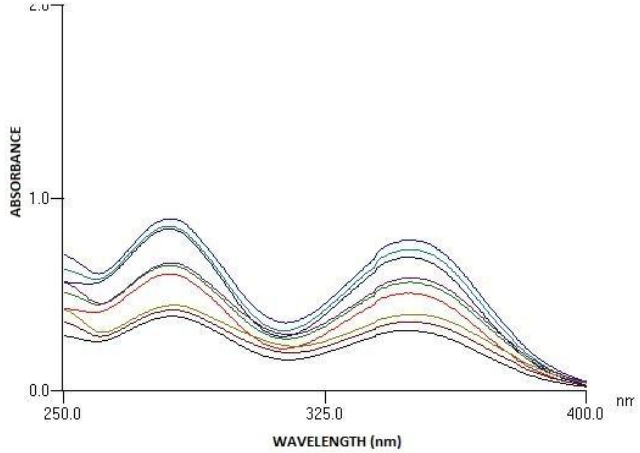

Fig. 3: Prediction spectra of Drotaverine Hcl and Mefenamic acid

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Table 1. Composition of calibration (Training set) for PLS and PCR methods

S.NO	Drotaverine Hcl			Mefenamic acid		
	Reference µg/ml	Predicted µg/ml		Reference µg/ml	Predicted µg/ml	
		PLS	PCR		PLS	PCR
1	2	2.08	2.08	6	6.024	6.024
2	2	2.07	2.07	12	12.05	12.04
3	2	2.06	2.06	18	17.92	17.93
4	2	2.05	2.05	24	23.92	23.94
5	2	2.03	2.03	30	30.08	30.09
6	4	4.05	4.05	6	6.024	6.024
7	4	4.04	4.04	12	12.059	12.06
8	4	4.04	4.04	18	17.92	17.93
9	4	4.04	4.04	24	23.92	23.92
10	4	4.02	4.02	30	30.08	30.08
11	6	5.96	5.96	6	6.02	6.02
12	6	5.95	5.95	12	12.05	12.04
13	6	5.94	5.94	18	17.94	17.93
14	6	5.93	5.92	24	23.92	23.92
15	6	5.92	5.92	30	30.07	30.08
16	8	8.01	8.01	6	6.022	6.022
17	8	7.99	7.99	12	12.048	12.048
18	8	7.98	7.98	18	17.92	17.92
19	8	7.97	7.97	24	23.92	23.92
20	8	7.96	7.96	30	30.07	30.07
21	10	10.04	10.04	6	6.02	6.02
22	10	10.03	10.03	12	12.04	12.04
23	10	10.02	10.021	18	17.92	17.92
24	10	10.01	10	24	23.92	23.92
25	10	9.99	9.99	30	30.07	30.07

Table 2. Composition of validation (prediction set) for PLS and PCR methods

S.NO	Drotaverine Hcl			Mefenamic acid		
	Reference µg/ml	Predicted µg/ml		Reference µg/ml	Predicted µg/ml	
		PLS	PCR		PLS	PCR
1	3	3.07	3.07	9	9.06	9.06
2	3	3.06	3.06	15	14.99	14.99
3	3	3.04	3.04	21	21.01	21.01
4	5	4.99	4.99	9	9.06	9.06
5	5	4.96	4.96	15	14.99	14.99
6	5	4.99	4.99	21	20.96	20.96
7	7	6.97	6.97	9	9.06	9.06
8	7	7.03	7.03	15	14.93	14.93
9	7	6.99	6.99	21	20.99	20.99

Selection of optimum number of factors and the spectral region

The most commonly employed validation criterion is to divide the dataset into two subsets, a calibration set and a validation set. The calibration model is calculated using the

calibration set. Then, the root mean square errors of calibration and validation, RMSEC - root mean square error of calibration and RMSECV - root mean square error of cross validation, are calculated using the calibration model under investigation to predict the samples in the calibration set and validation set, respectively.

For PCR and PLS methods, 25 calibration spectra were used for the selection of the optimum number of factors using the cross-validation with the leave-out-one technique. This allows modelling of the system with the optimum amount of information and avoidance of over-fitting or under-fitting. The cross-validation procedure consisted of systematically removing one of a group of training samples in turn and using only the remaining ones for the construction of latent variable factors and applied regression. The predicted concentrations were then compared with the actual ones for each of the calibration samples and the root mean square error of prediction (RMSEP) was calculated. The RMSEP was computed in the same manner each time, and then a new factor was added to the PCR and PLS models. The selected model was that with the smallest number of factors such that its RMSECV values were not significantly greater than that for the model, which yielded the minimum RMSECV. A plot of RMSECV values against the number of components indicates that the latent variable factor 3 was optimum for PCR and PLS models based on the RMSEC and RMSECV, respectively, for the estimation of the titled drugs. At the selected principal component of PCR and PLS, the concentrations of each sample were then predicted and compared with the known concentration and the RMSEP was calculated. The results are presented in Table. 3.

$$RMSECV = \sqrt{\sum_{i=y}^{N} \frac{\left(y_{ipred} - y_{iref}\right)^2}{N}} \qquad RMSEC = \sqrt{\frac{(y - y_{pred})^i (y - y_{pred})}{m - 1}}$$

Market Sample Analysis (Assay)

The proposed PLS and PCR methods were applied to the simultaneous determination of DROTA and MEFE in commercial tablets. Determination of six replicates was made. Satisfactory results were obtained for each drug in good agreement with the label claims. Assay spectra are shown in Fig. 4. The results are presented in Table. 4.

Precision

The method was found to be precise with six sample preparations for the quantification of DROTA and MEFE. The precision and intermediate precision variations were calculated in terms of relative standard deviation and the results were found to be less than 2.0% and the results are presented in Table. 5.

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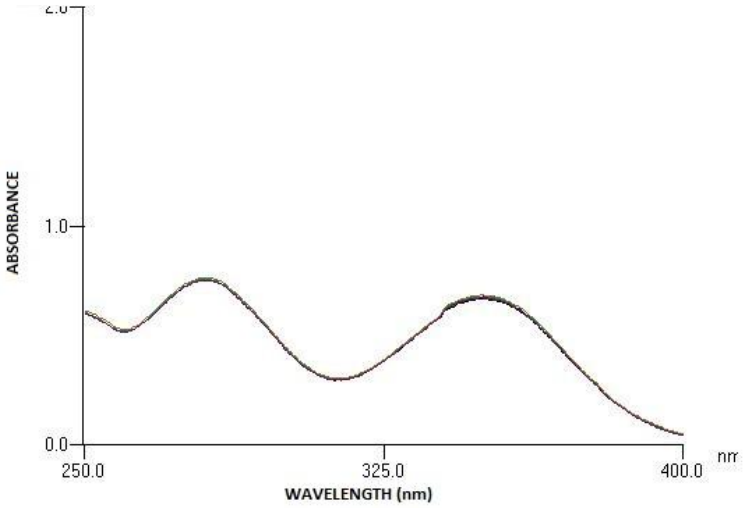

Fig.4: Assay spectra of Drotaverine Hcl and Mefenamic Acid

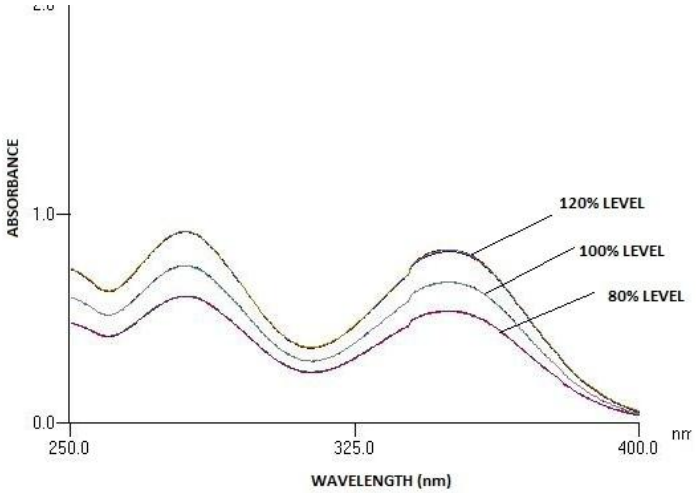

Fig. 5: Recovery spectra of Drotaverine Hcl and Mefenamic Acid

Table 3. Summary of Statistics in PLS and PCR methods for Drotaverine Hcl and Mefenamic acid in the mixture

Drug	RMSEP		RMSEC		r^2		Intercept		slope	
	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR
DR	0.0460	0.0438	0.0431	0.0354	0.9999	0.9998	0.0439	0.0009	0.9939	0.9998
MA	0.0539	0.0537	0.0641	0.0640	0.9999	0.9999	0.0048	0.0010	0.9997	0.9921

PLS – Partial Least Squares, PCR – Principal Component regression, RMSEP – Root Mean Square Error of Prediction, RMSEC – Root Mean Square Error of Calibration DR-Drotaverine Hcl and MA-Mefenamic acid.

Table 4. Analysis of tablet formulation

Formulation	Label claim	PLS mg/tab found*	PCR mg/tab found*
DROTIN-MF	Drota 80mg	81.77	81.75
	Mefe 250 mg	250.16	250.18

***Each value is a mean of six readings**

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Table 5. Precision Data

S.NO	System precision				Method precision			
	Drotaverine Hcl		Mefenamic acid		Drotaverine Hcl		Mefenamic acid	
	PLS µg/ml	PCR µg/ml	PLS µg/ml	PCR µg/ml	PLS % purity	PCR % purity	PLS % purity	PCR% purity
1.	6.00	6.00	18.13	18.13	102.16	102.15	99.69	99.69
2.	6.03	6.02	17.99	17.99	101.03	101.04	98.92	98.92
3.	6.04	6.03	18.13	18.13	101.90	101.89	99.76	99.76
4.	6.06	6.05	18.16	18.16	102.17	102.16	100.25	100.25
5.	6.05	6.05	17.99	17.99	101.60	101.50	100.10	100.10
6.	6.10	6.10	18.06	18.06	102.10	102.12	100.67	100.67
Mean	6.05	6.04	18.18	18.07	101.82	101.81	99.90	99.89833
S.D	0.0328	0.0343	0.07	0.07	0.4443	0.453784	0.5961	0.596
%RSD	0.5436	0.5677	0.41	0.413	0.4364	0.445717	0.5967	0.597
S.E	0.0133	0.0140	0.028	0.028	0.1813	0.1849	0.2433	0.2433
95%CI	±0.026	±0.027	±0.056	±0.056	±0.355	±0.362	±0.476	±0.476

S.D-standard deviation, %RSD-Relative standard deviation, S.E- Standard error, CI-confidence interval PLS-partial least square and PCR-principal component regression.

Recovery Studies

To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the pre-analysed formulation. (Standard addition technique) Recovery spectra are shown (Figure-5). The results are presented in Table. 6.

Table 6. Recovery Studies by PLS and PCR methods

% of Target		Drotaverine Hcl					Mefenamic acid				
		PLS			PCR		PLS			PCR	
		Added mg	Found mg	% Recovery	Found mg	% Recovery	Added mg	Found mg	% Recovery	Found mg	% Recovery
		4.8	4.799	99.97	4.801	100.02	14.4	14.42	100.13	14.41	100.06
		4.8	4.795	99.89	4.766	99.29	14.4	14.34	99.61	14.35	99.65
	80	4.8	4.816	100.3	4.812	100.25	14.4	14.45	100.34	14.46	100.41
		Mean	4.803	100.05	4.793	99.85	Mean	14.3	100.02	14.33	100.04
		SD	0.0115	0.217	0.024	0.501	SD	0.056	0.375	0.055	0.38
		%RSD	0.232	0.217	0.501	0.501	%RSD	0.397	0.375	0.384	0.38
		6	5.999	99.98	5.998	99.96	18	18.16	100.89	18.15	100.83
		6	5.937	98.96	5.938	98.96	18	18.01	100.04	18.02	100.1
	100	6	5.997	99.95	6.01	100.16	18	18.11	100.64	18.1	100.5
		Mean	5.977	98.71	5.982	99.69	Mean	18.09	100.53	18.09	100.47
		SD	0.035	0.58	0.038	0.642	SD	0.076	0.44	0.065	0.37
		%RSD	0.589	0.588	0.644	0.644	%RSD	0.422	0.44	0.362	0.37
		7.2	7.195	99.93	7.199	99.98	21.6	21.59	99.95	21.58	99.9
		7.2	7.199	99.98	7.198	99.97	21.6	21.68	100.37	21.66	100.27
	120	7.2	7.21	98.75	7.209	100.12	21.6	21.64	100.21	21.68	100.37
		Mean	7.201	100.13	7.202	100.02	Mean	21.63	100.18	21.64	100.18
		SD	0.007	0.696	0.006	0.084	SD	0.045	0.21	0.053	0.248
		%RSD	0.107	0.695	0.084	0.084	%RSD	0.208	0.21	0.245	0.248

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CONCLUSIONS

The most striking features of spectrophotometric method are its simplicity and rapidity without requiring time-consuming sample preparation. Chemometric calibration techniques in spectral analysis are widely used in quality control of drugs in mixtures and multi- component pharmaceutical formulations with overlapping spectra, as separation procedures in the drug determinations are not required. A comparative study of the use of PLS and PCR for the simultaneous spectrophotometric determination of Drotaverine Hcl and Mefenamic acid has been accomplished.

High percentage of recovery shows that the methods are free from interference of the excipients used in the commercial formulation. Results also showed that the developed methods can be applied to a routine analysis, quality control of mixtures and commercial preparations containing these drugs.

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